

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

- 1-6. (Canceled)
7. (Currently Amended) A method for the absolute quantification of a target nucleic acid in a sample comprising the steps of:
- (a) ~~determination of~~ determining the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions ~~as claimed in claim 1; by:~~
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the internal or external standard;
    - (ii) amplifying the target nucleic acid and the internal or external standard under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of the internal or external standard, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the internal or external standard used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the internal or external standard from said non-linear continuously differentiable function;

- (b) ~~amplification of~~ amplifying the target nucleic acid contained in the sample and ~~of~~ the internal or external standard under ~~the same~~ said defined reaction conditions;
  - (c) ~~measurement of~~ measuring the amplification of the target nucleic acid and that of the internal or external standard in real time; and
  - (d) ~~calculation of~~ calculating the original copy number in the sample by correcting ~~correction of~~ the copy number derived from step (c) with ~~the aid of~~ the amplification efficiencies determined in step (a).
8. (Currently Amended) A method for ~~the~~ quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:
- (a) ~~determination of~~ determining the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions as ~~elaimed in claim 1;~~ by:
    - (i) preparing a dilution series of the target nucleic acid and a dilution series of the reference nucleic acid;
    - (ii) amplifying the target nucleic acid and the reference nucleic acid under defined reaction conditions and measuring the amplification in real-time;
    - (iii) setting a defined signal threshold value;
    - (iv) determining, for each dilution of the target nucleic acid and for each dilution of reference nucleic acid, the cycle number at which the signal threshold value is exceeded;
    - (v) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid and the reference nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and
    - (vi) calculating the amplification efficiency of the target nucleic acid and the reference nucleic acid from said non-linear continuously differentiable function;

- (b) ~~amplification of~~ amplifying the target nucleic acid contained in the sample as well as the reference nucleic acid contained in the sample under ~~the same~~ said defined amplification conditions;
  - (c) ~~measurement of~~ measuring the amplification of the target nucleic acid and that of the reference nucleic acid in real time; and
  - (d) ~~calculation of~~ calculating the original ratio of target nucleic acid and reference nucleic acid in the sample by ~~correction of~~ correcting the ratio derived from step (c) with ~~the aid of~~ the amplification efficiencies determined in step (a).
9. (Currently Amended) A method for ~~the relative~~ quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:
- (a) ~~preparation of~~ preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
  - (b) ~~amplification of~~ amplifying the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acid ~~being measured~~ acids in real-time;
  - (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
  - (d) determining the cycle numbers  $C_p$  ~~at~~ to which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
  - (e) determining a continuously differentiable function of the  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the ~~determined~~  $C_p$  values determined in step d) as a function of the logarithm of the amounts used of reference nucleic acid;

- (f) determining ~~determination~~ of the Cp values of the target nucleic acid and reference nucleic acid in ~~the~~ a sample to be analysed as well as in a calibrator sample;
  - (g) assigning ~~assignment~~ of the Cp values measured in step f) to particular values of the functions determined in step e);
  - (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
  - (i) determining ~~determination~~ of the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.
10. (Currently Amended) A method for ~~the relative~~ quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:
- (a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;
  - (b) amplifying ~~amplification~~ of the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, and measuring the amplification of the nucleic acids ~~being measured~~ in real-time;
  - (c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;
  - (d) determining the cycle numbers Cp at which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;
  - (e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the Cp values determined in step d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the ~~determined~~ Cp values determined in step d);

- (f) determining the  $C_p$  values of the target nucleic acid and reference nucleic acid in ~~a the~~ sample to be analysed as well as in a calibrator sample;
  - (g) assigning ~~assignment of~~ the  $C_p$  values measured in step f) to particular ~~function~~ values of the functions determined in step e);
  - (h) calculating the quotients of the function values from step g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and
  - (i) determining ~~determination of~~ the ratio of the two quotients from step h) as a measure of the original amount of target nucleic acid contained in the sample to be analysed.
11. (Currently amended) The method of claim 10, wherein the continuously differentiable functions from step e) are determined with ~~the aid of~~ a polynomial fit ~~preferably of the~~  $3^{rd}, 4^{th}, 5^{th}, 6^{th}$  or  $7^{th}$  degree.
12. (Currently Amended) The method of claim 10, wherein the amplified nucleic acids are detected with ~~the aid of~~ at least one fluorescently-labeled hybridization probe.
13. (Currently Amended) The method of claim 12, wherein the amplified nucleic acids are detected with ~~the aid of~~ FRET hybridization probes, molecular beacons, or TAQMAN<sup>®</sup> probes.
14. (Currently Amended) The method of claim 10, wherein the amplified nucleic acids are ~~Currently with a DNA-binding dye, preferably SybrGreen I.~~